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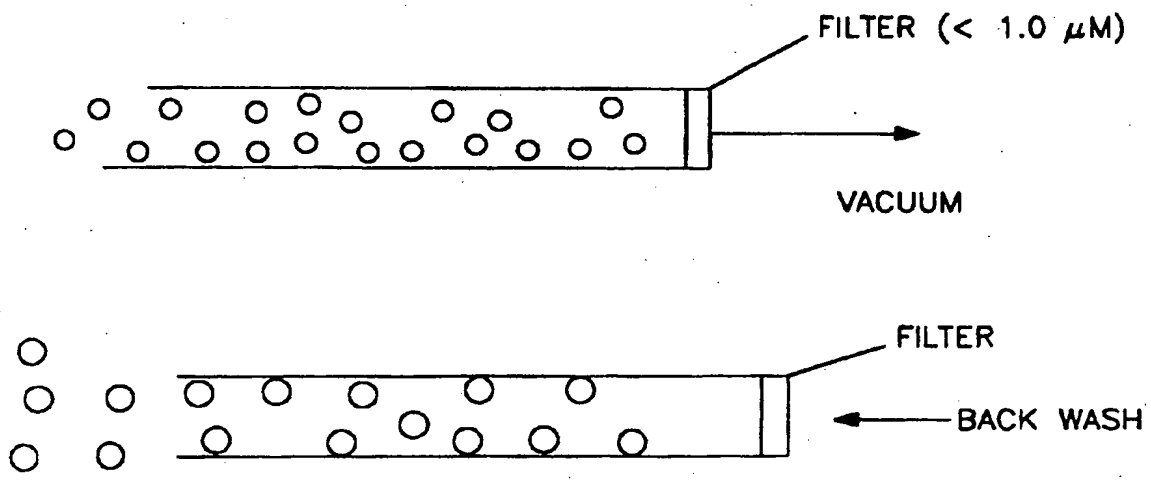

(57) Abstract

Electrophoretic media and devices comprising said media are provided, wherein said media is capable of being reversibly converted in response to an applied stimulus from a first state of low viscosity to a second state of high viscosity, which is continuous and electrophoretically sievable. The subject media comprise a continuous fluid phase, a polymeric composition, such as discrete microgel particles that can be swollen and collapsed in response to the applied stimulus, and optionally a binder. The subject media find use in electrophoretic devices comprising a gel holder, such as slab gel devices, column and capillary devices. Among other advantages, the subject media provide for ease of preparation, superior gel formation and improved resolution of electrophoretically separated components.

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SEPARATION MEDIA FOR USE IN GEL ELECTROPHORESIS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of Application Serial No. 08/241,048 filed on May 10, 1994, which application is a continuation-in-part of application serial no. 07/971,956 filed November 5, 1992, now abandoned.

INTRODUCTION

Technical Field

The field of this invention is separation media for use in electrophoresis.

Background of the Invention

Gel electrophoresis has become an increasingly indispensable tool in biotechnology and related fields. The ability to separate molecules by means of size, shape and charge has added numerous opportunities to identify specific compounds, determine purity, and allow for isolation of a compound in a relatively pure form. A variety of analytical techniques are predicated on the use of gel electrophoresis for the separation and analysis of the various components of interest that may be present in a particular sample. For example, gel electrophoresis may be used to identify a compound, where the components of a complex mixture are first separated and then subsequently identified by using markers such as antibodies, DNA probes or the like. Furthermore, gel electrophoresis may also be used in the determination of the molecular weights of components in a sample.

Electrophoretic devices may comprise a variety of gel holders, such as slab holders, columns, microchannels, capillaries and the like. Each type of device has advantages and disadvantages. For example, devices comprising slab gels provide for the possibility of running multiple samples side by side. However, the resolution and speed that may be achieved in these devices is limited due to constraints on the voltage that may be applied to the gel during electrophoresis. In contrast, separations in gel filled capillaries may be run at voltage gradients which are much higher than those which may be used in traditional slab platforms, due to the excellent heat transfer ability of the capillary, providing for rapid resolution of sample components, coupled with reproducibility of results and high levels of sensitivity.

Despite the growing use of electrophoretic devices in an increasing number of applications, problems continue in the preparation of homogeneous, stress free gels in these devices. For example, polymerization induced shrinkage and appearance of bubbles in the gel are common problems encountered in the preparation of gel filled capillaries for capillary gel electrophoresis. Although numerous alternative acrylamide monomers and cross-linkers have been proposed with the objective of improving some aspect of gel performance, none of these media have, as yet, addressed these of gel preparation. (For a general review of gel electrophoresis media, *see* Righetti *et al*, J. Chromatography, 638, 165 (1993)).

Thus, there is a continuing need for the development of a gel electrophoresis medium which would provide for improved ease in gel preparation, where the prepared gels would be free from bubble, cracks or other irregularities and provide for good separation of sample components during electrophoresis. Also of interest would be a gel which would provide for the above ease in preparation and yet, once formed, retain mechanical rigidity for further analysis of the separated components in the gel.

Relevant Literature

Patents of interest which disclose various media for use in electrophoresis include U.S. Patent 5,055,517; U.S. Patent 5,164,057; U.S. Patent 3,956,273; U.S. Patent 5,143,646; and U.S. Patent 5,149,419. U.S. Patent 4,732,930 discloses

ionic isopropylacrylmide gels which exhibit volume changes in response to solvent composition, temperature, pH or ion composition and U.S. Patent 5,100,933 discloses a method of causing a discontinuous volume change in a gel in response to changes in metal ion concentration, whereby the gel is an ionized, cross-linked polyacrylamide gel.

The use of hydrophilic gel beads in isoelectric focusing is reported in Radola *et al*, *Biochim Biophys.* Acta, 386, 181 (1974).

Peacock & Dingman, *Biochemistry*, 7(21)668 (1968) and Bode, *Analytical Biochemistry*, 83,204, 1977 report the preparation and use of agarose-acrylamide composite gels for electrophoresis. The use of entangled polymers as media for gel electrophoresis is reported in: Bode, *FEBS Lett.*, 65, 56, 1976; Tietz et al, *Electrophoresis*, 7, 217, 1986; Grossman & Soane, *Biopolymers*, 31, 1221, 1991; Grossman & Soane, *J. Chromatography*, 559, 257 (1991); Bode, *FEBS Lett.*, 65, 56 (1991); Zhu *et al.*, *J. Chromatography*, 480, 311 (1989); Chin & Colburn, *Am. Biotech. Lab.*, 7, 16 (1989); Tietz *et al. Electrophoresis*, 7, 217 (1986)).

The response of various gel electrophoresis media to changes in temperature, pH, ionic strength, and/or cosolvent concentration is reported in: Heskins & Guillet, *J. Macromol. Sci. - Chem.*, A2 (8), 1441, (1968); Taylor & Cerankowski, *J. Polym. Sci., Polym. Chem. Ed.*, 13, 2551, (1975); Hirokawa & Tanaka, *J. Chem. Phys.*, 81, 6379, (1984); Ilavsky *et al*, *Polym. Bull.*, 7, 107, (1982); and Boyde *J. Chrom.*, 124, 219 (1976).

SUMMARY OF THE INVENTION

Novel electrophoretic media and devices comprising said media are provided, where said electrophoretic media are capable of reversibly changing, in response to an applied stimulus, from a first flowable state of low viscosity to a second electrophoretically sievable state of high viscosity, where in the second state the media are characterized by having sieving properties suitable for electrophoresis. The applied stimulus may be one or a combination of stimuli, such as a change in temperature, pH, or solution composition of the media. In a preferred embodiment, the subject medium comprises discrete microgel particles which undergo a volume

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change in response to an applied stimulus. Where desired, the subject media may further comprise a binder which provides mechanical rigidity to the media. The subject media find use a in electrophoretic devices comprising gel holders, such as slab gel holders, columns and capillaries.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A, 1B, and 1C are schematics of filling (Figure 1A), swelling (Figure 1B), and deswelling and flushing capillaries (Figure 1C).

Figures 2A and 2B are schematics illustrating physically cross-linked systems. Thin lines are hydrophilic polymers and thick lines are hydrophobic polymers. The shaded areas represent physically bonded regions, via the hydrophobic salting out phenomenon.

Figures 3A and 3b are schematics of ionic association, forming physical cross-links: 3A, dissociated state; 3B, associated state.

Figures 4A and 4B are schematics of chelated association, forming physical cross-links: 4A, dissociate state; 4B, associated state.

Fig. 5 is a schematic showing a reversibly solubilized system according to the invention.

Fig. 6A and 6B show magnified photographs of the microgel dispersion made according to Example 3 at 35°C and at 30°C.

Fig. 7 shows the electropherogram of the analysis shown in Example 7.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Novel electrophoretic media and devices comprising said media are provided. The electrophoretic media are capable of reversible conversion from a first flowable state of low viscosity to a second electrophoretically sievable state of high viscosity in response to an applied stimulus. The applied stimulus may be one, or a combination of, a change in temperature, pH, or solution composition of the medium. In a preferred embodiment, the subject media comprise discrete microgel particles whose volume changes in response to the applied stimulus. The subject media may further comprise a binder which provides for mechanical rigidity to the

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gel media. The subject media find use in a variety of electrophoretic devices, including devices comprising slab gel holders, column, capillaries and the like.

Media which may be used in the subject devices are characterized by being capable of a reversible transition, in response to an applied stimulus, from a first state of low viscosity to a second state of high viscosity. In the first state, the subject media are characterized by being readily flowable, pourable and/or pumpable. The subject media in the first state are characterized by having a low sieving capability and thus are not suited for use in electrophoresis in this state. Generally, the viscosity of the subject media in the first state will range from 1 to 10 to 100,000 cP, usually from 30 to 50,000 cP, and more usually from 50 to 10,000 cP. The low viscosity of the first state provides for rapid and substantially complete filling of the gel holder of the device in which the media are employed. The media comprise a continuous fluid phase, a polymer composition and, optionally, a binder.

In the second state of high viscosity, the media become substantially continuous, electrophoretically sievable, matrices. The continuous nature of the media in this second state provide the media with substantial sieving properties, thereby making the media suitable for use in electrophoresis. In this second state, the viscosity of the media will be at least 100 % greater than the viscosity of the media in the first state. The viscosity of the media in this second state will be at least about 1000 cP, usually at least about 5,000 cP and more usually at least about 10,000 cP, where the viscosity may be as high as 5×10^6 cP or higher, where the media becomes a solid, elastic like gel solid mass that is no longer flowable, pourable or pumpable.

The transition of the subject media from the first to the second state occurs in response to an applied stimulus. The applied stimulus may be any number of changes in the conditions of the media, including changes in the temperature, pH, fluid phase composition, *e.g.* a substitution of ionic species of the fluid phase from monovalent to divalent species, an increase in the percentage of organic polar solvents, *e.g.* ethanol or acetone, in the fluid phase, *etc.*, of the media. The transition from the first to the second state will be reversible, so that upon modulation of the stimulus responsible for the transition from the first to the second

state, the media will return to the first state. For example, if the media responds to a change in temperature, the transition from the first to the second state may be caused by raising the temperature of the media. If the temperature of the media is then lowered to its original temperature, the media will return from the second state of higher viscosity to the first state of the lower viscosity. For changes in the liquid phase, volatile components may be evaporated.

In one embodiment of the invention, the subject media will be an aqueous media comprising a solution of discrete, chemically cross-linked microgel particles or beads. The microgel particles will have a size distribution ranging from no smaller than about 10 nm, usually at least about 30 to 50 nm, and no larger than about 2 mm. The optimal size of the particles in a particular medium will depend on the gel swelling ratio and dimensions of the electrophoresis channel or chamber of the device in which the medium is employed. The discrete microgel particles will be swellable, such that the volume of the particles will increase substantially, usually by at least about 50%, in response to the applied stimulus. In aqueous media comprising discrete microgel particles, the weight concentration of the microgel particles in the solution will range between about 1% and 50%, preferably not more than about 25 %. The fluid phase of the aqueous medium comprising the gel particles may be a buffered solution of various pH and ionic strength, pure deionized water depending on the specific application, comprise organic or inorganic solvents, agents such as surfactants, and the like. In media according to this preferred embodiment, the media may be characterized as a dispersion of discrete microgel particles, where the media will be readily pourable or flowable. Upon application of a stimulus to the media, the particles will swell, resulting in a medium which may be characterized as a substantially continuous matrix that is free of void spaces.

The subject microgel particles may be prepared from any suitable monomer components capable of polymerizing into microgel particles having the above characteristics. The subject gel particles or microgels may be prepared from a variety of monomers including: N-adamentylacrylamide, N-benzylacrylamide, N-benzylmethacrylamide, N-cyclohexylacrylamide, N,N'-diethylacrylamide, dodecylmethacrylamide, N-isobornylacrylamide, N-methymethacrylamide, diacetone

acrylamide, N-[3-(dimethylamino) propyl] acrylamide, methacrylamide, and (1-naphthyl methyl) methacrylamide and the like. Cross-linkers that may be used in the preparation of the subject microgel particles from the above monomers included: N,N'-bis (1,2-ethylene) dimethacrylamide, N,N'-ethylenebisacrylamide, N,N' -hexamethyl bisacrylamide, N,N'-methylenebisacrylamide, methylenebismethacrylamide, N,N'-nonomethylenebisacrylamide, N,N'-octamethylenebisacrylamide, NN'-(isopropylidene) bisacrylamide, NN'-trimethylenebisacrylamide, piperazine diacrylamide, N,N'bisacrylylcystamine, and N,N'-diallyltartardiamide and the like.

The subject gel particles may be prepared using any convenient method, including suspension polymerization and precipitation polymerization. In suspension polymerization, an aqueous solution of a hydrophilic monomer is dispersed in a continuous hydrophobic medium using a surface-active substance which promotes the formation of water-in-oil emulsions. The polymerization is then initiated with water-soluble initiators, *e.g.* sorbitan monostearate and the like. In preparing the subject microgel particles by suspension polymerization, the emulsifier is first dissolved in a suitable organic medium, such as toluene or xylene. The aqueous monomer is then introduced with agitation, resulting in a crude suspension. The crude suspension is then homogenized to decrease the average droplet size and increase the emulsion stability. Polymerization is then initiated using any convenient means, such as raising the temperature of the media, introducing a polymerization initiator to the medium and the like. The resultant particles may be small as 30 nm, where the amount of surfactant used in the method will affect the resultant particle size.

Instead of suspension polymerization, the subject microgel particles may be prepared by precipitation polymerization. In precipitation polymerization, polymerization of the monomer is carried out by introducing a polymerization initiator into an aqueous medium comprising the monomer, where the temperature of the medium is above the lower critical solution temperature (LCST) of the polymer, *i.e.* the temperature above which the polymer will precipitate out. Thus, in this method an aqueous medium of water soluble monomers is prepared and the

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temperature of the prepared medium is maintained above the LCST of the particular polymer to be prepared, where LCSTs of polymers are known in the art. Generally the temperature will be at least about 50 °C, and usually at least about 70 °C. Following preparation of the aqueous medium comprising the monomer, a polymerization initiator, *e.g.* potassium persulfate, is introduced and the polymerization reaction is allowed to go to completion under conditions of mild agitation. The size of the resultant microgels will depend on the conditions of polymerization, such as temperature or intensity of agitation, with more intense agitation resulting in smaller sized particles. The resultant particles may be as small as 50 nm.

In either of the above discussed preparation methods, the resultant microgel particles may be purified using any convenient means, such as centrifugation and washing, and the like. Thus, the resulting medium comprising the polymerized microgels may be centrifuged to separate the microgels rom the suspending medium, followed by washing in water. The process may be repeated as desired to increase the purity of the microgels.

In an alternative embodiment of the subject invention, instead of employing a media comprising discrete, reversibly swellable, microgel particles, the media may comprise a reversibly cross-linkable system of polymers. Reversibly cross-linkable polymer systems that may be used in the subject media include copolymers of hydrophilic and hydrophobic monomers. These copolymers form blocky structures of hydrophilic and hydrophobic regions, where the hydrophobic regions can be physically bonded and released in response to an applied stimulus, such as a change in the temperature and ionic strength of the buffer solution comprising the copolymers. See Figs. 2A & 2B. Thus, in the first state the copolymers will be linear and, upon appropriate stimulus, will transform to the second state of coalesced polymers which form a network having sieving properties suitable for electrophoresis.

The subject media could also comprise a polymeric component which comprises soluble, un-crosslinked polymers in which the viscosity of the media is responsive to an applied stimulus, where cross-linking of the polymers does not

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occur. Thus, although a cross-linking of polymers has not occurred, the applied stimulus, such as a change in temperature, changes the viscosity and sieving properties of the media.

Instead of having a copolymer as shown in Figs. 2A & 2B, one may have a graft copolymer of two distinct polymers, where a first polymer forms a scaffolding structure and a second polymer, engrafted onto the first polymer, reversibly changes its structure or orientation with respect to the first polymer in response to an applied stimulus, as described above. An exemplary graft polymer would be one in which a first or scaffolding polymer forms a structure of large pores with minimal sieving capability. The second or switchable polymer would be engrafted onto the first polymer so that in the first state prior to application of the stimulus, the engrafted second polymer strands would align adjacent to the first polymer thereby occupying a minimal portion of the pores of the first polymer. Upon application of the stimulus, the second polymer changes position so as to occupy a substantial portion of the pores of the first polymer, thereby significantly changing the sieving properties of the graft polymer medium. In this embodiment, the first or scaffolding polymer that provides a structure to the media would generally be one which reversibly changes from a first to a second state at a different applied stimulus from the stimulus which causes the second, engrafted polymer to transition from the first to the second state. For example, one could have the first polymer which is flowable at a first elevated temperature and forms a solid, porous matrix at a second temperature. Over the range of temperatures at which the first polymer sets into the gel like state, the second, engrafted polymer could change position, thereby filling the the pores of the first polymer and changin the sieving properties of the media. Thus, by modulating the temperature further to the point where the engrafted polymer changes position relative to the first polymer, the engrafted polymer may then change position so as to substantially fill the pores of the first polymer. The change in position of the engrafted polymer may be proportional to the change in temperature, providing for control over the degree of filling of the pore size of the media and the possibility of dynamically changing the pore size of the media, as will be discussed further below.

Alternatively, the media may comprise polymers having functional groups that cross-link or dissociate in response to the exchange of monovalent ions for divalent ions in the solution in which the polymers are present. See Figs. 3A & 3B. For example, a buffer containing zinc divalent ions may be added to the gel media comprising the polymers, thereby displacing sodium salts present in the media and resulting in the cross-linkage of the polymers. Additional polymers that may be reversibly cross-linked include polymers comprising functional groups, such as hydroxyl or amino groups, which crosslink in response to the presence of chelating agent, such as titanium ion and the like. See Figs. 4A & 4B. In this system, the cross-link is reversible via addition of strong acid or oxidizer. In yet another alternative embodiment of the subject invention, the subject media may be ones which comprise polymers capable of reversibly solubilizing, such that in the first state the polymers are insoluble and in the second state the polymers are soluble, resulting in a continuous network of polymers. See Fig. 5.

The subject media may also comprise discrete microgel particles in conjunction with soluble, uncrosslinked polymers, *e.g.* linear or branched polyacrylamides, *etc.*, where this composite media is also capable of reversibly converting from a first to second state. Thus, in the first state where the particles are collapsed so as to occupy minimal volume, the concentration of linear polymer in the interstices between the particles will be reduced, resulting in a medium with no, or insufficient, sieving capability for electrophoresis. Following application of a stimulus and swelling of the particles, the volume of the interstices between the particles will decrease, with a concomitant increase in the concentration of linear polymer in the interstices. This increase is accompanied by an increase in the sieving capability of the media so a point where the media is useful for electrophoresis, *i.e.* is an electrophoretically sievable medium.

Where desired, the subject media may further comprise a binder which is capable of imparting mechanical strength and rigidity to the media in the second state. Inclusion of the binder in the gel media makes possible subsequent identification procedures, *e.g.* staining, blotting *etc.*, of the components in the gel, *i.e.* further analysis of the electrophoretically separated sample components in the

gel, where the gel comprising the electrophoretically separated sample components is removed from the gel holder of the device in which electrophoresis has taken place. Thus, following electrophoresis, an intact slab or column gel may be removed from the gel holder of the device and then stained. Where the media comprises a binder in addition to the polymer, *e.g.* the microgel particles or other polymers described above, the binder will be present in amount ranging from about 0.5 % to 10%, usually .5 % to 5 %, and more usually 0.5 to 3 %. Any binder which is capable of providing the desired mechanical rigidity to the media and reversibly setting into a rigid matrix in response to thermal modulation may be used in the subject media. Binders for use in gel electrophoresis are known in the art and include agarose, gelatin, and the like.

The subject media find use in a variety of electrophoretic devices or platforms comprising gel holders which serve as electrophoresis or separation chambers. Electrophoretic devices in which the subject media find use include slab devices, column devices, microchannel devices, capillary devices and the like.

In preparing and using electrophoretic devices according to the subject invention, the first step is the introduction of the medium into the gel holder of the device. The medium will be introduced into the gel holder in the first state of low viscosity. Since the medium is readily pourable and flowable in the first state, it will substantially fill the gel holder without voids, bubbles or other irregularities. Although the media will substantially fill the gel holder, it will not have sieving properties suitable for electrophoresis since it is in the first state of low viscosity.

Following introduction of the media into the gel holder, a stimulus will then be applied to the medium which results in the transition of the medium from the first to the second state. The stimulus may be applied using any convenient means, such as raising the temperature of the medium by increasing the applied voltage across the medium or introducing an agent into the medium, such as a chelator, by electrophoresis. Following the transition from the first to the second state, electrophoresis of a sample in the device may then be carried out by introducing the sample into the gel medium and applying a sufficient voltage gradient across the gel. Any sample amenable to electrophoresis may be employed, from samples which

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comprise a single component to samples that comprise a plurality of components. If desired, following electrophoresis, the medium may be returned to the first state of low viscosity through application of the appropriate stimulus, *e.g.* lowering the temperature of the medium.

Although the subject media are suitable for use in any electrophoretic device comprising a gel holder, the media are particularly suited for use in capillary gel electrophoresis devices. In preparing a capillary for gel electrophoresis with a medium comprising discrete microgel particles according to the subject invention (with the following description equally applying to the other media described above, e.g. media comprising the graft copolymers and media comprising the uncrosslinked polymers), the medium is first introduced into the separation chamber or interior space of the capillary using any convenient means, such as injection or suction by vacuum. When the medium is introduced in the interior of the capillary, it will be in the first state of low viscosity, where the microgels are in the collapsed state. At the end of the capillary, a filter whose pore size is less than the diameter of the collapsed particles may be placed so that only the suspending liquid can pass through the filter. See Fig. 1A. In this way, the capillary can be filled under media conditions where the media is maintained in the first, flowable state, i.e. where the discrete microgel particles remain collapsed. The diameter of the microgels in this collapsed state will depend on the gel swelling ratio of the particular microgel particles and diameter of the capillaries, but will usually range from about 5 μ to 250 μ.

Once the medium is introduced into the capillary, the medium can be converted to the second state of high viscosity, where the microgel particles swell to produce a continuous matrix of polymer. See Fig. 1B. In other words, the total particle volume of each particle in the second state is such that the particles deform against one another to become a continuous phase. The result is a capillary which is filled with particles only and contains minimal void space, *i.e* a capillary that is free of voids or interstices between the particles. The resultant medium has sieving properties which are suitable for electrophoresis.

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Electrophoresis of a sample in the medium filled capillary may then be performed. During electrophoresis, the media may be maintained in a constant state, so as to provide for constant sieving characteristics during electrophoresis of a sample. Alternatively, the conditions of the media may be modulated during electrophoresis, thereby by changing pore size and sieving properties of the media. Thus, one can provide for dynamic sieving conditions during electrophoresis, where the sieving conditions may be optimized for a particular sample to achieve more efficient resolution of the separated sample components. For example, the conditions may be set in the media and the sample may be partially separated on the media. After partial separation, the conditions may be changed, for example, by swelling to increase the pore size, whereby more defined separations may be obtained. In addition to changing the sieving properties of the media by stepwise changes in the applied stimulus, one may also apply a gradual change in the stimulus to achieve a dynamic change in the sieving properties of the media. For example, one could apply a temperature gradient to the medium so that the pore sizes of the media gradually change at a defined rate during electrophoresis.

Following electrophoresis, the capillary can be easily flushed of medium by changing the medium conditions back to the conditions at which the capillary was filled, thereby providing for reuse of the capillary gel holder. See Fig. 1C. For example, the temperature may be changed to the temperature used in the capillary filling stage so that the particles collapse. The pH and or solution composition of the medium may also be changed to synergistically achieve deswelling of the particles. For example, one can change the temperature to a point at which the gel spheres shrink and then pass a buffer solution having a low pH or low ion concentration though the gel by electrophoresis. Alternatively, with the acrylamide monomers referenced above, a ketone such as acetone or low alcohol such as methanol or ethanol is injected into the capillary so the particles are collapsed to a minimum size for easy flushing.

In addition to providing for improved homogeneity of gels which are free from defects, the subject media provide for several unique possibilities following electrophoresis of the sample. The ability to change the pore size of the continuous

medium during electrophoresis through additional modulation of the medium's conditions, *e.g.* modulation of the temperature, pH, composition of the fluid phase, as described above, of the medium, provides for the possibility of removing components from the gel following electrophoresis so that the gel medium may be reused for subsequent separations. For example, following a separation, the particles could be swollen to increase pore size so that upon application of a sufficient voltage gradient across the gel, substantially all of the separated sample components run through the gel. Alternatively, where one is working with a media comprising the graft copolymers, the engrafted polymers could be switched to the state where they are aligned with the scaffolding polymer, thereby maximizing the pore size of the medium allowing for substantial removal of the all of the sample components from the medium. After substantially all of the sample components have run through the gel or electrophoretic medium, the pore size of the particles may then be reduced for subsequent use of the gel medium in electrophoresis.

Furthermore, in media comprising a binder in addition to the polymer, following electrophoresis a section of the gel comprising the separated sample component of interest can be excised from the remainder of the gel. Following excision of the component of interest, the separated gel section can then be subjected to an appropriate stimulus so that the medium returns to its first state of low viscosity, whereby the separated component may be readily extracted from the excised section. This procedure provides for an efficient way to remove separated sample components from a gel.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Example 1 Preparation of Cross-linked micro-gel particles.

A. Suspension Polymerization

In inverse emulsion polymerization an aqueous solution of a hydrophilic monomer is dispersed in a continuous hydrophobic oil medium using a surface-active substance which promotes the formation of water-in-oil emulsions. The polymerization is then initiated with either oil-soluble or water-soluble initiators. As far as the end-product of the reaction is concerned, it is clear that inverse lattices are less stratified or flocculate more readily. Continuous and gentle agitation is needed to maintain these lattices as colloidal dispersion indefinitely.

Acrylamide was used as the water soluble monomer and N,N'-methylene-bis-acrylamide as a cross-linker to prepare a $T_{10}C_2$ solution, where:

$$\%T = \frac{\text{grams of acrylamide} + \text{grams of cross-linker}}{\text{Total volume}} \times 100$$

$$\%C = \frac{\text{grams of cross-linker}}{\text{grams of acrylamide} + \text{grams of cross-linker}} \times 100$$

Sorbitan monostearate (SMS) was used as an emulsifier. The emulsions were formed by dissolving 0.875 g of SMS in 7 ml of o-xylene and adding $T_{10}C_2$ solution (the aqueous monomer solution) with stirring for about 3 to 4 hrs. The temperature was controlled at about 50°C during polymerization. The crude emulsions were homogenized to decrease the average droplet size and to increase the emulsion stability.

After complete polymerization, the final gel particles were cleaned by centrifuging several times with deionized water. The gel particles were not monodisperse, however they could be filtered to a certain size range, for example, using a 1 μ filter to collect particles less than 1 μ.

B. Precipitation Polymerization

Precipitation polymerization was carried out above the LCST of the system. The following reagents were combined:

N-isopropylacrylamide:	4.9 g
N,N'-methylene-bis-acrylamide:	0.1 g
Potassium persulfate:	0.2 g
deionized water:	200 ml

The system temperature was controlled at about 70°C. The final gel particles are reasonably monodisperse.

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Example 2 *Changing of gel swelling equilibria by changing ambient conditions.*

The gel swelling equilibria can be changed by changing ambient conditions, i.e. temperature, pH, solvent. Gel disks were prepared by casting acrylamide/N,N'-methylene-bisacrylamide gels in glass tubes (ID = 6.4mm). The resultant gel disk dimensions were about 6 mm in diameter and about 13 mm in thickness.

After preparing the gel disks, each sample was blotted with laboratory tissue to remove surface water, and weighed. The swelling capacity was determined as the mass ratio of swollen gel to the gel prepared. The volume of the gel as cast (not the dry gel) is denoted by V_o. In each swelling capacity measurement, the diameter and the length of each gel disk was also measured. By observing the response of the gel disks to changes in ethanol or acetone concentration of the medium in which the gel disks were placed, it was found that the volume ratio (V/V_o) of the gel particles decreased from 1.2-1.4 to 0.2-0.3 with increasing concentrations of ethanol or acetone. It was also found that the volume ratio (V/V_o) changed in response to changes in pH and temperature.

Example 3 *Suspension polymerization of temperature sensitive poly(NIPA) microgels.*

300ml toluene and 2.5g Span 80 were mixed in a round bottom reaction flask equipped with a nitrogen inlet and an overhead stirrer. An aqueous premix of 6.8g N-isopropylacrylamide, 0.07g N,N'-methylene bisacrylamide and 0.1g ammonium persulfate in 50g water was added to the organic phase while stirring. The mixture was purged with nitrogen for 15 minutes, following which 1.5ml TEMED was added to initiate polymerization. The reaction was allowed to proceed for 3 hours under nitrogen atmosphere, following which the stirring was stopped, the resulting hydrogel particles settled in the reaction kettle, and the top organic layer was removed. The hydrogel particles were isolated by centrifugation and redispersion in distilled water, repeated three times. Figures 6A & 6B show a magnified photograph of the resulting microgel dispersion above the LCST (at 35°C) and below the LCST (at 30°C).

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Example 4 Precipitation polymerization of temperature-sensitive poly(NIPA) microgels and viscosity transition.

N-isopropylacrylamide (4.9g) and N,N'-methyl bisacrylamide (0.1g) were dissolved in 190g water in a round bottom reaction flask equipped with a condenser, a nitrogen inlet and an overhead stirrer. The solution was purged with nitrogen and the temperature adjusted to 70°C. 10ml of potassium persulphate solution (containing 0.2g potassium persulfate) were added to initiate polymerization. The reaction was allowed to proceed for 24 hours under nitrogen while stirring.

The resulting hydrogel microspheres were isolated and concentrated by centrifugation, followed by redispersion in distilled water. This centrifugation/ redispersion process was repeated three times, and in the final redispersion step the polymer concentration was adjusted was adjusted to 9% by weight of solution. The viscosity of this dispersion was measured between 4 and 35°C. The results are provided in Table 1.

Table 1. Viscosity Transition of NIPA Microgel Dispersion

Temperature (°C)	Viscosity (cP)
4	9×10^5
5	8×10^5
6	8×10^5
10	7×10^5
15	6×10^5
20	4×10^5
25	3×10^5
29	8×10^4
30	5×10^4
31	2×10^4
32	8×10^3
33	1×10^3
34	800
35	600

The results demonstrate that as the temperature of the media increases, the viscosity descreases.

Example 5 *Viscosity change resulting from surfactant concentration.*

Poly(NIPA) microgel solution from Example 4 was adjusted to 8% by weight. Varying amounts of sodium dodecyl sulfate (from 0 to 2wt%) were added to this solution, and the viscosity of the resulting mixtures were measured. The results are provided in Table 2.

Table 2

% SDS	Viscosity (CP) at Temp. = 20.7 °C	Viscosity (CP) at Temp. = 25 °C
0	3×10^4	7×10^3
0.5	6×10^4	*
1.0	1×10^5	7×10^4
1.5	1.6×10^5	1.2×10^5
2.0	2.5×10^5	2×10^5

The results demonstrate that increasing the concentration of the surfactant SDS in the medium increases the viscosity of the medium at each temperature studied.

Example 6 *Suspension polymerization of copolymer microgel system.*

3.3ml N,N'-dimethylacrylamide, 3.3ml N,N'diethylacrylamide, 0.067g N,N'-methylene bisacrylamide, 0.1 ammonium persulfate and 7g Span 80 were dissolved in 45ml water in a round bottom reaction flask equipped with a nitrogen inlet and an overhead stirrer. While stirring, approximately 1/3 of a total of the 250ml toluene was added to the aqueous premix creating a thick emulsion. The remaining 2/3 of the 250ml toluene was added three minutes later. The reaction mixture was purged with nitrogen for 15 minutes, after which 1.5ml TEMED was added to initiate polymerization. The reaction was allowed to proceed for 3 hours under nitrogen. Approximately 400ml of acetone was added to the mixture causing phase separation with the hydrogel microspheres precipitating in the lower phase. The upper, organic-rich phase was removed, and distilled water was added to redisperse the concentrated microgels. Residual organics were distilled off in a rotary evaporator.

Example 7 *Capillary electrophoresis separation of dsDNA in copolymer microgel system.*

An optical window was formed in a fused silica capillary (250 microns ID, 350 microns OD) by burning off the polyimide coating with an electrically heated filament. The capillary was internally coated with linear polyacrylamide by the method of Hjerten (*J. Chromatorgr.*, 347, 191, 1985). Copolymer microgel solution from Example 6 adjusted to 9% polymer by weight in 1X TBE buffer, was loaded into the capillary by heating the gel and capillary to 90°C and drawing the microgel solution in by vacuum. 27.2cm of capillary (20.2cm effective length) was loaded into the cartridge of a Beckman PACE 2100 capillary electrophoresis, system. A 10 base pair dsDNA ladder, 0.05 mg/ml, was electrokinetically injected at 4 kV for 15 seconds, cathode at the injector end. After injection, the capillary was run at 2 kV (74 V/cm) with detection by UV at 254 nm. Figure 7 displays the electropherogram showing resolution of the 10 to 100 base pair peaks.

Example 8 *Preparation of an agarose-microgel composite matrix.*

0.05 g agarose and 10ml of copolymer microgel from Example 6 (having a polymer concentration of 9%) were mixed and stirred at 80°C for 15-20 minutes. The solution was then poured into a horizontal electrophoresis unit. After cooling to room temperature for 1/2 hour, the mixture formed a rigid (non-viscous) gel that could be handled.

Example 9 *Chaotropic switch of linear, water soluble polymers suitable for electrophoresis.*

Aqueous solutions of sodium sulfate were made ranging in concentration from zero to 15 percent by weight. Hydroxyethylcellulose was added to make the solutions 1% by weight polymer. After agitation, the viscosities were measured and are shown in Table 3. At sodium sulfate concentrations less than 8%, the solutions were clear and viscous. At concentrations greater than 10%, the solutions were a very low viscosity suspension of the polymer powder.

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Table 3
Chaotropic Switch. 1% (w/w) HEC Solution with Added Sodium Sulfate

% Sodium Sulfate	Viscosity (cP)
0	2×10^4
5	2×10^4
6.7	2×10^4
8.35	2×10^4
10	7×10^3
11.7	2
13.35	2
15	2

Polyethylene oxide (MW 900,000) was dispersed similarly at 4% by weight in solutions of varying sodium sulfate concentration. At concentrations above 7%, the polymer coagulated into a solid phase. Below this, the solutions were clear and viscous. Both polymers, when made into small microgel particles, will exhibit a viscosity switch due to varying the concentration of a chaotropic agent such as sodium sulfate.

Example 10 Reversible cross-linking of a derivatized cellulose with a titanium chelate.

A stock solution of 0.5% (w/w) of a cis-diol derivatized hydroxyethyl-cellulose solution was prepared and the viscosity measured as shown in Table 4. 18ml of this solution was acidified with 200mg of glacial acetic acid and 100mg of triethanolamine titanate chelate was added with vigorous shaking. The system gelled in seconds and the viscosity was measured. 300mg of concentrated sulfuric acid was added and mixed into the gel with a spatula following by continued mixing on a rocking stage. After 30 minutes the viscosity was measured again.

Table 4. Reversible Viscosity Switch of Derivatized Cellulose with Titanium Chelate

Media	Viscosity (cP)
Original solution	394
After addition of chelate	444000
After addition of sulfuric acid	232

This example system can be loaded at low viscosity, the titanate added and the system gelled for electrophoresis. After use, the system can be lowered in viscosity by acid and removed.

Example 11 *Solvent switch of polyacrylamide microgels in an acetone/water system.*

A range of acetone/water blends were prepared. Polyacrylamide microgel powder (45-90 microns diameter fully hydrated) was added to each to make 7% by weight solutions. After agitation for several hours, the solution viscosities were measured. The results are provided in Table 5.

Table 5
Polyacrylamide Microgels, 7% loading, Viscosity v. %Acetone

% Acetone (w/w)	Viscosity (cP)
0	1.9×10^6
5	1.9×10^6
10	1.9×10^6
15	3.0×10^4
20	6.0×10^2
25	36

As shown in Table 5, in pure water the viscosity was at least 1.5 million cP, while at 25% acetone by weight the viscosity had dropped to 36 cP.

It is evident from the above examples and discussion that improved electrophoretic devices are provided where the gels present in these devices will be homogeneous, stress free gels which are free of irregularities and provide for high resolution of electrophoretically separated components. The subject media overcome

the traditional problems of preparing homogenous stress-free gels in electrophoretic devices, such as polymerization induced shrinkage and the appearance of bubbles, cracks and other irregularities in the gel. The subject media also provide for new methods of using electrophoretic devices, such as the ability to optimize the pore size of a gel during a separation, as well as the ability to remove separated components following an electrophoretic separation, thereby allowing for the possibility of gel reuse.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. An electrophoretic device comprising an electrophoretic medium comprising: (1) a composition capable of reversibly converting, in response to an applied stimulus, from a first flowable state to a second electrophoretically sievable state; and (2) a binder.

2. The device according to Claim 1, wherein said composition is discrete particles of a cross-linked N-substituted polyacrylamide of a size in the range of 10 nm to 2 mm in an aqueous medium; and in said second state said particles are swollen and form a continuous gel.

3. The device according to Claim 1, wherein said binder is agarose.

4. The device according to Claim 1, wherein said stimulus is a change in at least one of: temperature, pH, or fluid phase composition of said electrophoretic medium.

5. An electrophoretic device comprising an electrophoretic medium comprising a binder and a composition capable of reversibly converting in response to an applied stimulus from a first flowable state to a second electrophoretically sievable state, wherein in said first state said composition is a dispersion of discrete particles of a cross-linked N-substituted polyacrylamide of a size in the range of 10 nm to 2 mm in an aqueous medium, and in said second state said particles are swollen and form a continuous electrophoretically sievable gel matrix.

6. The device according to Claim 5, wherein said device comprises a capillary gel holder.

7. The device according to Claim 5, wherein said device comprises a slab gel holder.

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8. The device according to Claim 5, wherein said device comprises a column gel holder.

9. An electrophoretic medium comprising a binder and a composition capable of reversibly converting in response to an applied stimulus from a first flowable state to a second electrophoretically sievable state, wherein in said first state said composition is a dispersion of discrete particles of a cross-linked N-substituted polyacrylamide of a size in the range of 10 nm to 2 mm in an aqueous medium, and in said second state said particles are swollen and form a continuous electrophoretically sievable gel matrix.

10. An electrophoretic medium comprising a fluid phase and a graft copolymer, wherein said composition is capable of reversibly converting in response to an applied stimulus from a first flowable state to a second electrophoretically sievable state.

11. An electrophoretic medium comprising an uncrosslinked polymer and a composotion capable of reversibly converting in response to an applied stimulus from a first flowable state to a second electrophoretically sievable state, wherein in said first state said composition is a dispersion of discrete particles of a cross-linked N-substituted polyacrylamide of a size in the range of 10 nm to 2 mm in an aqueous medium, and in said second state said particles are swollen and form a continuous electrophoretically sievable gel matrix.

12. A method for preparing an electrophoretic device for use in gel electrophoresis, said method comprising:

introducing into a gel holder of said electrophoretic device an electrophoretic medium comprising: (1) a composition capable of reversibly converting in response to an applied stimulus from a first flowable state to a second continuous electrophoretically sievable gel state and (2) a binder; and

applying said stimulus to said medium;

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whereby said medium converts from said first to said second state.

13. The method according to Claim 12, wherein said medium in said first state is a dispersion of discrete particles of a cross-linked N-substituted polyacrylamide of a size in the range of 10 nm to 2 mm in an aqueous medium; and in said second state said particles are swollen and form said continuous gel state.

14. The method according to Claim 12, wherein said stimulus is a change in at least one of: temperature, pH or fluid phase composition of said medium.

15. A method of performing electrophoresis on a sample with an electrophoretic device prepared according to the method of Claim 12, said method comprising:

introducing a sample into said electrophoretic medium; and

electrophoresing said sample;

whereby said sample is electrophoresed, resulting in a gel comprising electrophoretically separated sample components.

16. The method according to Claim 15, wherein the conditions of said medium are modulated during said electrophoresis, whereby the sieving properties of said medium are changed.

17. A method of removing an electrophoretically separated component of a sample electrophoretically separated according to the method of Claim 15 from a gel, said method comprising:

excising a portion of said medium comprising said separated component from said medium while said medium is in said second state;

returning said medium to said first state; and

extracting said separated component from said excised portion;

whereby said separated component is separated from said gel.

18. A method for removing separated components from an electrophoretic device prepared according to the method of Claim 12 without removing said medium from said gel holder, said method comprising:

modulating the conditions of said medium to increase the pore size of said medium; and

applying a voltage gradient across said medium sufficient for substantially all of said separated components to be removed from said gel.

19. A method of identifying at least one sample component of an electrophoretically separated sample according to the method of Claim 15, wherein said gel is rigid, said method comprising:

removing said gel comprising said electrophoretically separated sample from said gel holder; and

identifying said at least one sample component.

FIG. 1 A

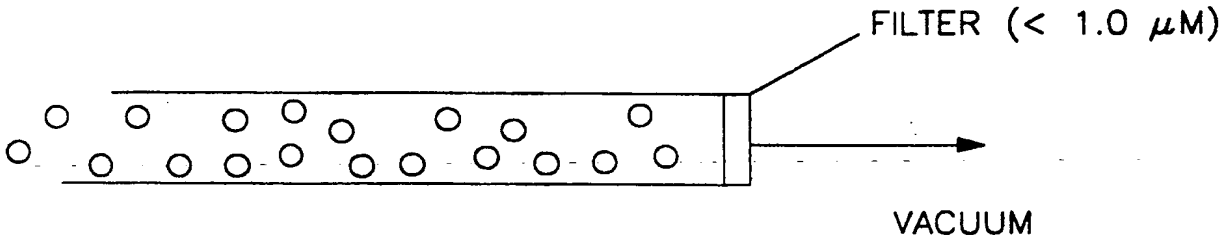


FIG. 1 B

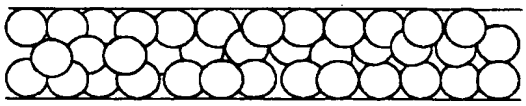


FIG. 1 C

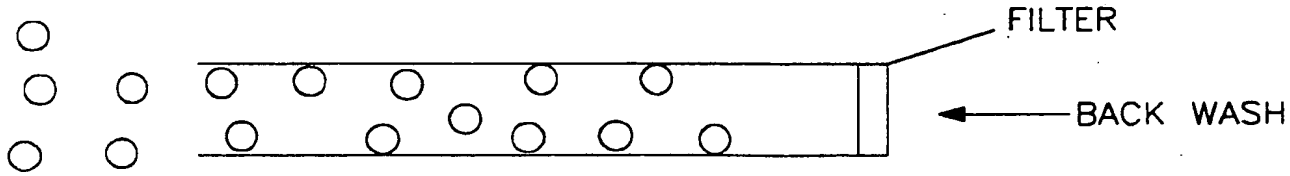


FIG. 2A

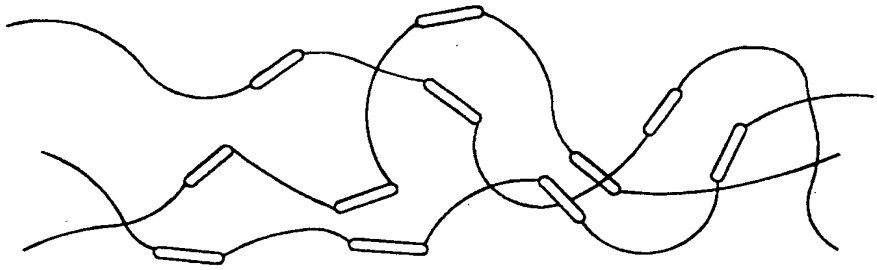
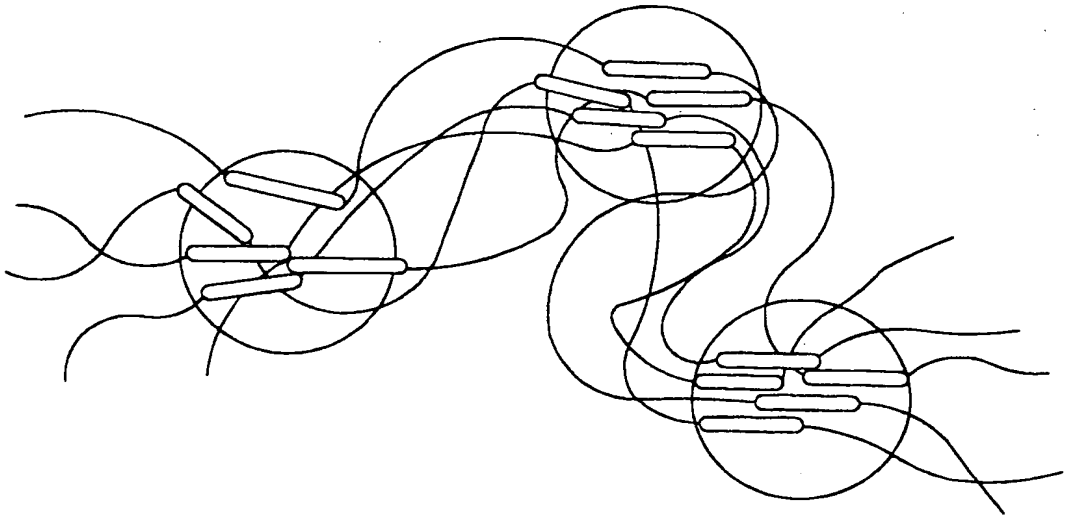


FIG. 2B



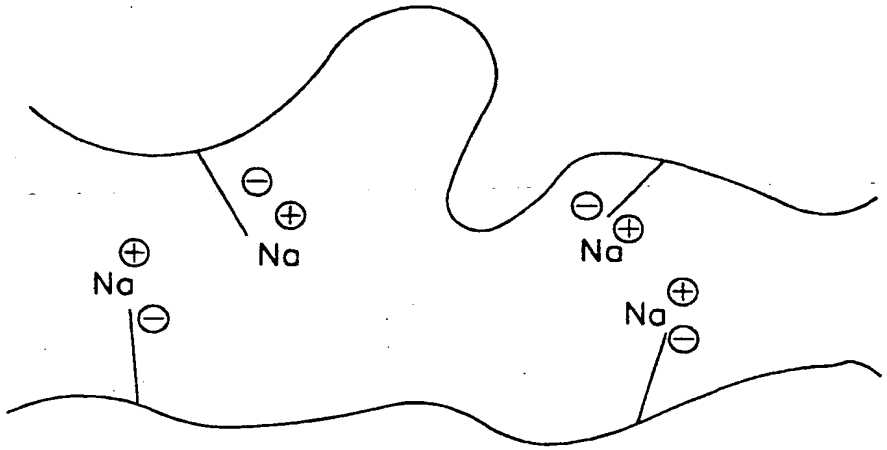


FIG. 3A

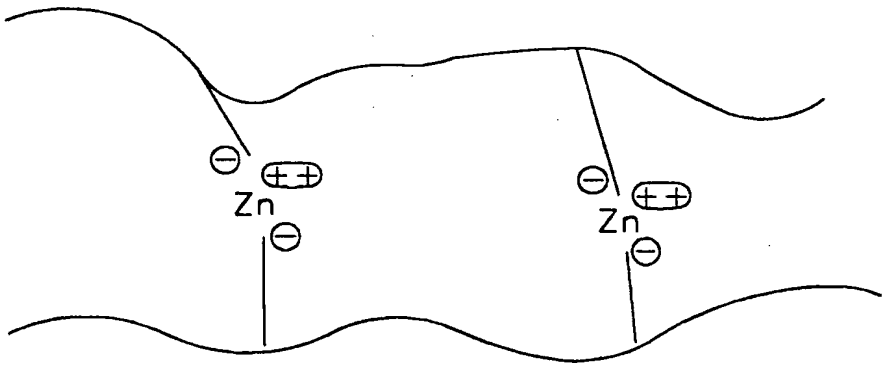


FIG. 3B

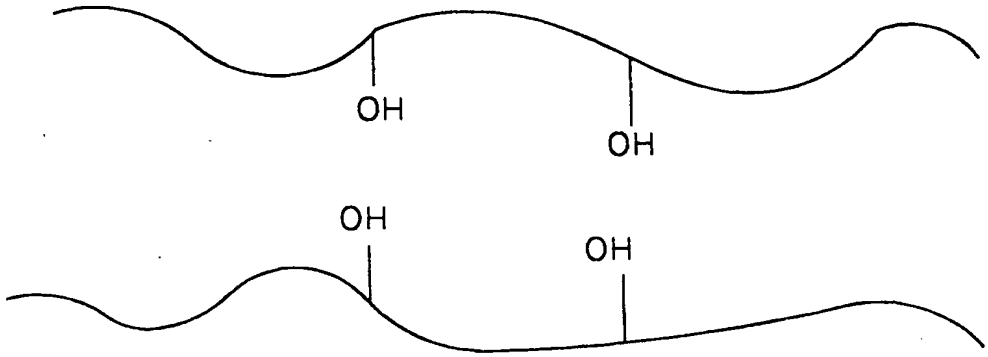


FIG. 4A

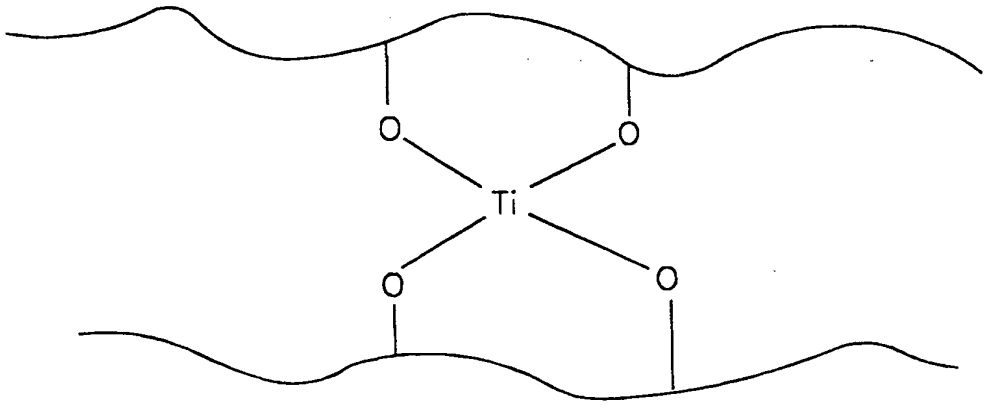
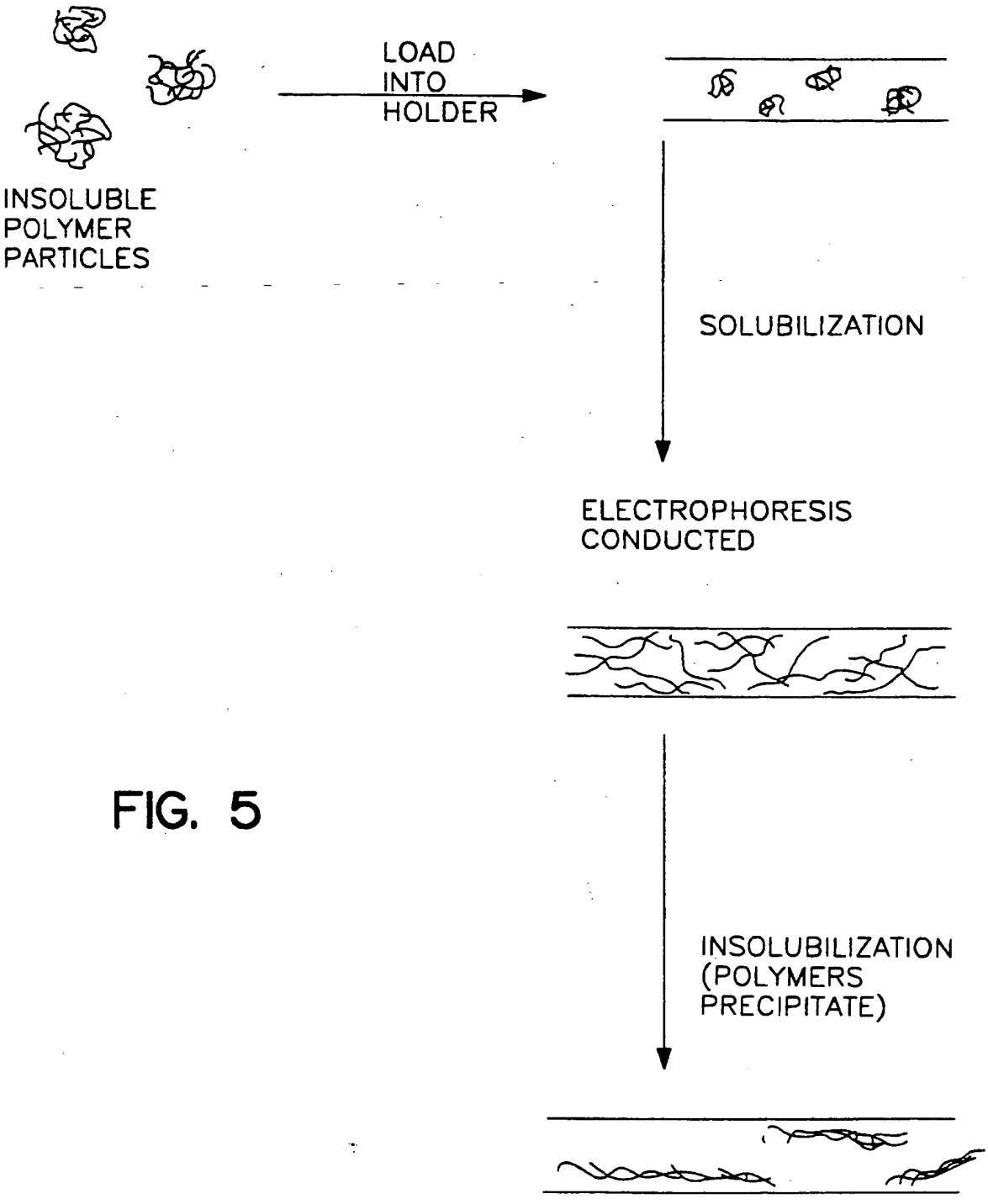


FIG. 4B


LOAD
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PRECIPITATE)

FIG. 5

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30°C

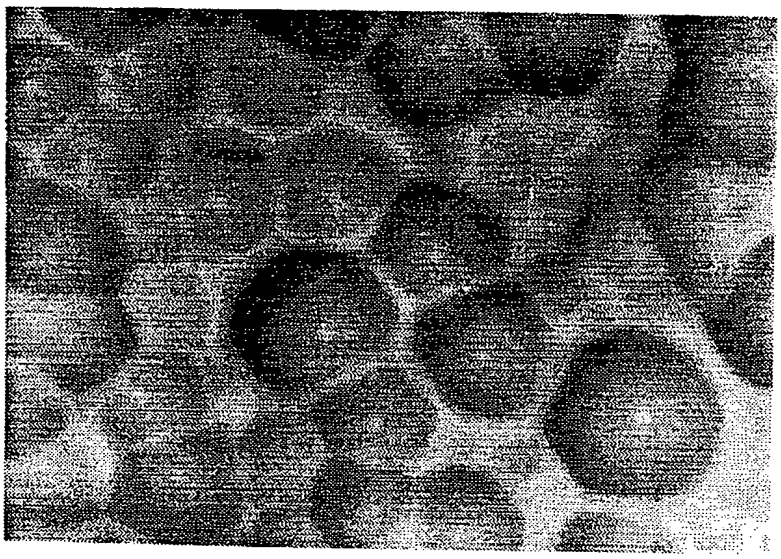

FIG. 6A

35°C

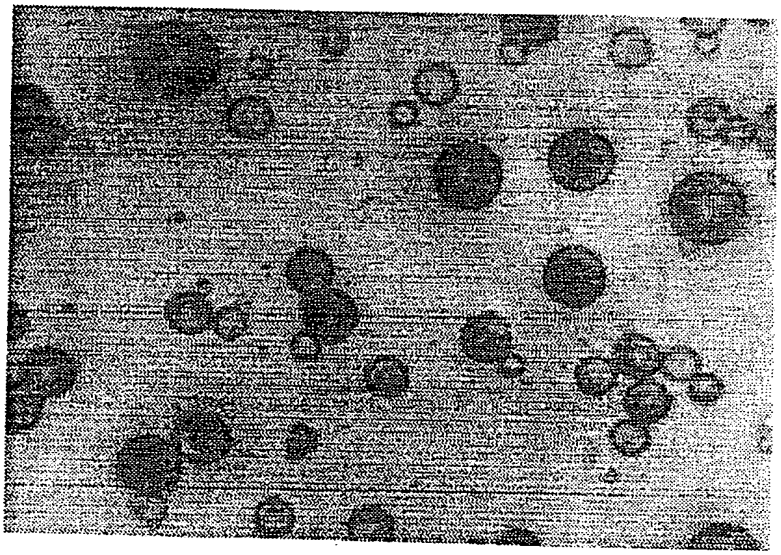

FIG. 6B

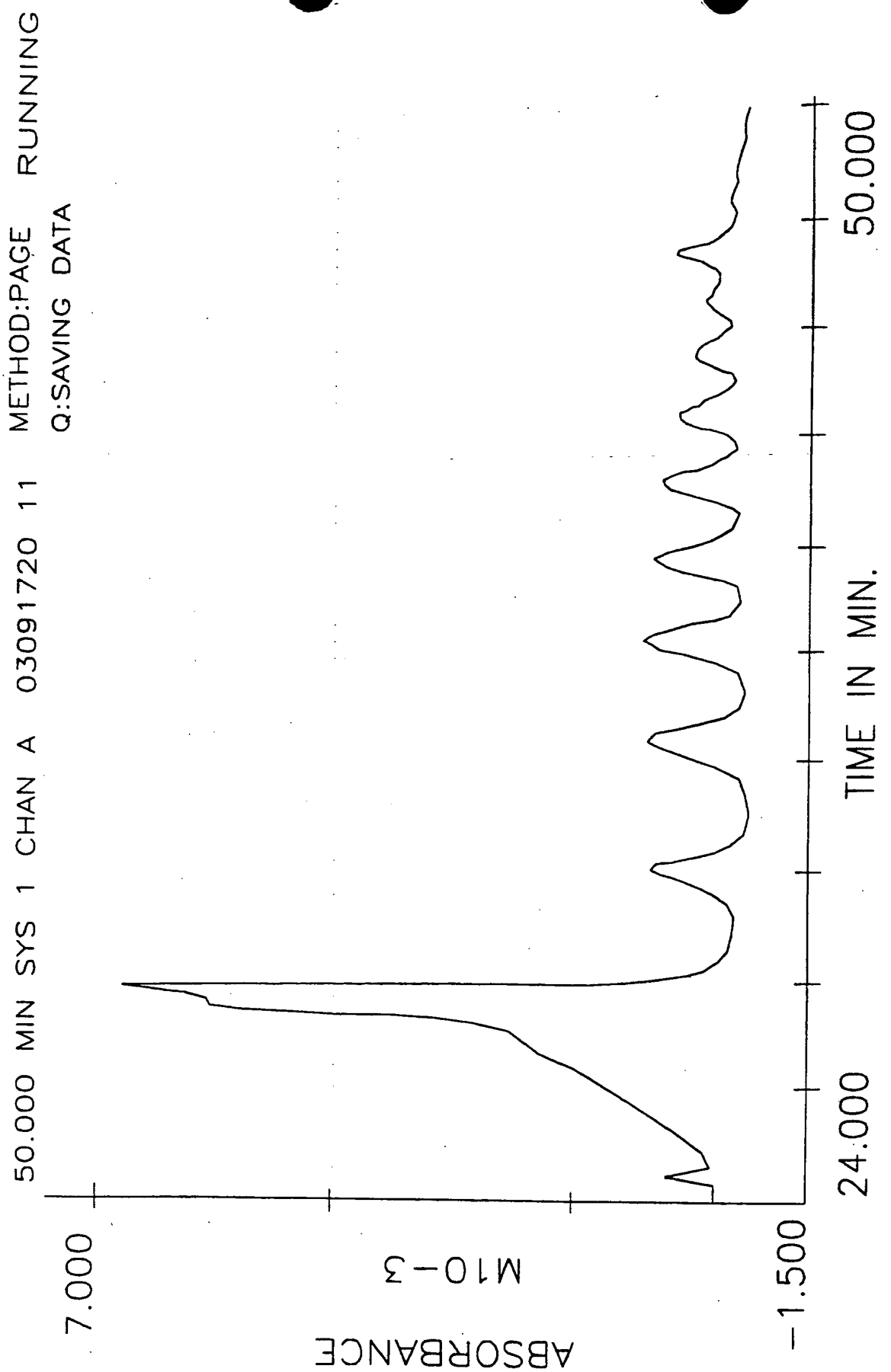

FIG. 7

DOCID: <WO 9530782A1 | >

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/06080

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C25B 7/00, 9/00
US CL : 204/299R, 182.8
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 204/182.8 , 299R, 252/315.1; 526/303.1, 306 524/555

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS: swelling, electrophoresis, gel, particles.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A, 5,135,627 (Soane) 04 August 1992, col. 4, lines 1-10; col. 6, lines 41-42.	1-19
Y	US,A, 5,019,232 (Wilson et al.) 28 May 1991, col. 3, lines 43-45; Col. 4, lines 44-47; Col. 5, lines 16-23, claims 9, 13-16.	1-19
Y	US,A, 4,732,930 (Tanaka et al.) 22 March 1988, abstract; col. 1, lines 17-19.	1-19
Y	US,A, 5,238,545 (Yoshioka et al.) 24 August 1993, Col. 5; lines 34-44; Col. 3, lines 11-16 and lines 66-68.	1-19
Y	US,A, 5,225,062 (Yoshioka et al) 06 July 1993, col. 5, lines 34-46; Col. 3, lines 11-16 and line 67 to Col. 4, line 1-2.	1-19

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
09 JULY 1995

Date of mailing of the international search report
10 AUG 1995

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/06080

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A, 4,863,613 (Johnson et al.) 05 September 1989, Col. 7, lines 27-39, 49-50, 58-60; col. 8, lines 12-15; claims 1, 3-6.	1-19
A	US,A, 5,242,491 (Mamada et al.) 07 September 1993.	
A	WO, A, 90/07978 (Numson et al) 26 July 1990.	
Y	US,A, 5,100,933 (Tanaka et al.) 31 March 1992, abstract; col. 1, lines 29-31.	1-19

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